Best regards from Norm Horowitz

Perspectives

Anecdotal, Historical And Critical Commentaries on Genetics

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The Sixtieth Anniversary of Biochemical Genetics

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MAY, 1996 marks 60 years since the publication in GENETICS of the paper that opened modern biochemical genetics (BEADLE and EPHRUSSI 1936). The authors, GEORGE W. BEADLE and BORIS EPHRUSSI, had published some preliminary reports of their experiments on the transplantation of imaginal discs in Drosophila, but this was their first full paper on the subject. A direct line leads from this publication to the production of nutritional mutants in Neurospora and bacteria and to all that followed from that.

The two authors, both in their thirties, had met at Caltech in 1934 and had agreed to launch this investigation in order to throw light on the mechanisms of gene action in development. The original plan had been to transfer imaginal discs from Drosophila larvae to a tissue culture medium, where, it was hoped, the discs would continue to develop. With this idea in mind, the site of the investigation was moved from Caltech to EPHRUSSI's laboratory at the Institut de Biologie in Paris, which was equipped for tissue culture studies (Figure 1). The discs did not do well *in vitro,* however, and the two researchers decided instead to transplant them to the body cavity of other larvae, a technique that had been used successfully in the moth Ephestia by CASPARI (1933). In the larval environment, the discs developed and yielded the results to be described below.

The inspiration for these experiments was an early observation by STURTEVANT (1920), who found that, in gynandromorphs of Drosophila, genetically *vermilion* eyes behaved nonautonomously, *i.e.,* they developed not *vermilion,* but wild-type, eye color, if the other parts of the fly were genetically wild type for the *vermilion* gene. This nonautonomy, with its hint that in some cases the phenotypic defect of a mutant could be rectified, suggested to BEADLE and EPHRUSSI that the transplantation method might be applied to the study of gene action in Drosophila. It is not clear how much influence CASPARI's earlier findings with Ephestia had on this decision, but there was probably some.

Interest in the biochemistry of gene action appeared shortly after the rediscovery of Mendelism and led to some notable early researches. The best known and most brilliant of these were by LUCIEN CUÉNOT and ARCHIBALD GARROD, who, respectively, studied inherited traits in mice and men. These authors were the first to link gene mutations to specific biochemical defects (see WAGNER 1989). Their work was followed by studies by others on the inheritance of coat colors in mammals and anthocyanins in plants (for references, see STURTEVANT 1965). These early investigations had been largely forgotten by geneticists by the time BEADLE and EPHRUSSI began their work. This neglect was due only partly to the fact that genetics took a different turn after the highly fruitful chromosome theory came into existence. At least as important must have been the fact that higher animals and plants, with their long generation times and inherent complexities, were too difficult and unpromising a material for studies of the biochemistry of gene action. Even Drosophila, as we shall see, was only marginally useful for this purpose. It was not until geneticists discovered microorganisms that a science of biochemical genetics could come into existence.

In their 1936 paper, BEADLE and EPHRUSSI studied the fate of eye discs from 26 different eye-color mutants of Drosophila after their implantation into the body cavity of larvae of the same or a different genotype. The discs developed into adult eyes, which, because they were detached from the optic nerve, failed to evert, but which were otherwise normal eyes. Their pigmentation, in particular, developed normally.

Of the 26 mutants, just two, *vermilion* and *cinnabar,* proved to be nonautonomous, *i.e.,* they developed wild-type pigmentation when grown in a wild-type host. The authors inferred that, in both cases, a diffusible substance needed for production of normal pigmentation was supplied by the host to the mutant disc. The effect of the mutation had been to prevent formation of the substance. They found, furthermore, that a different substance was required by each mutant. This was shown by reciprocal implants in which a *cn* disc developing in a *v* host remained *cn,* but a *v* disk in a *cn* host became wild type. This result showed that *cn* mutants produce what they called the v^+ substance, but *v* mutants do not

FIGURE 1.—BORIS EPHRUSSI (left) and GEORGE W. BEADLE (right) transplanting imaginal discs; Paris, 1935. Courtesy of the Archives, California Institute of Technology.

make the cn^+ substance. A simple explanation of these facts, they suggested, assumes that the v^+ substance is a precursor of the cn^+ substance in a reaction chain, so that a block in the synthesis of the former (a mutation from v^+ to v) also blocks synthesis of the latter. Mutation from cn^+ to *cn,* however, blocks only the synthesis of the cn^+ substance.

In later papers, they often referred to the two substances as "hormones" because in implanted flies they are formed in one place and used in another. As will be seen, it was eventually found that the two substances are actually precursors of the brown component of the Drosophila eye pigment. The other component is red. The duplex nature of the pigment was shown by German workers in the late 'thirties.

In their 1936 paper, BEADLE and EPHRUSSI also included the claret gene in the reaction sequence, as a precursor of the v^+ substance. Thus,

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance}$$

The evidence for a ca^+ substance was indirect, since claret eye discs develop autonomously in wild type. It derived from the observation that wild-type eye discs transplanted to *claret* develop "*claret*-like" pigmentation. This was interpreted to mean that the eye needs a factor made elsewhere in the fly to develop full wild-type coloration. *claret* lacks this factor; hence, the ca^+ substance.

This interpretation soon led to logical difficulties that increasingly required *ad hoc* explanations for newer observations. The latter included the findings that the *claret* effect was variable in degree and that it was observed in implants to mutants other than *claret,* in particular those known to be deficient in the v^+ substance. Before long, the existence of the ca^+ substance was called into question (BEADLE *et al.* 1938), and finally the idea was abandoned (CLANCY 1942). The *claret* story illustrates difficulties that are perhaps inevitable when critical judgements are based on visual estimates of subtle differences in shades of color.

The validity of the *vermilion* and *cinnabar* findings was not affected by the difficulties with *claret,* and it became the goal of the BEADLE and EPHRUSSI groups to identify the v^+ and cn^+ substances. EPHRUSSI was still in Paris, and BEADLE was now at Stanford University, where he had been joined by EDWARD TATUM. The two groups succeeded in their quest, but the story has a curious ending, as will be seen.

Several discoveries made in the two laboratories greatly facilitated the identification. It was found that v^+ substance was effective when fed to larvae or injected into them, making it no longer necessary to implant eye discs. Then it was found that addition of tryptophan to the fly food led to partial restoration of the brown pigment; in other words, tryptophan supplied in this way showed v^+ activity. Next it was discovered that the

effect of tryptophan was caused by bacteria growing in cultures to which this amino acid had been added. TATUM then isolated a Bacterium sp. that produced large amounts of a substance with v^+ activity from tryptophan. He set about isolating the active factor and by 1940 had obtained a crystalline preparation which he identified as a compound of L-kynurenine with sucrose (TATUM and HAAGEN-SMIT 1941). The compound showed high v^+ activity; acid hydrolysis removed the sucrose and left all the activity in the kynurenine moiety. This, then, was the long-sought substance.

The odd twist to the story is that kynurenine had been identified as the v^+ substance some months earlier by BUTENANDT *et al.* (1940), German chemists who had been studying insect eye colors. Learning that the active compound was a bacterial product formed from tryptophan, they simply tested known metabolites of the amino acid for v^+ activity. L-kynurenine was active. The paper by TATUM and HAAGEN-SMIT confirmed this finding. BUTENANDT's group later found that the cn^+ substance is 3-hydroxy-L-kynurenine (BUTENANDT *et al.* 1949), and they eventually showed that the brown pigment is formed by condensation of two molecules of hydroxykynurenine. The v^+ and cn^+ substances are thus precursors of the pigment.

In subsequent investigations, the enzymes determined by the *vermilion* and *cinnabar* genes were identified. Their activities are missing from the respective mutants (see GHOSH and FORREST 1967). Further interesting details of this work are reviewed in a later publication by COCHRAN (1975).

The *vermilion-cinnabar* case differed importantly from the mutations studied earlier by CUÉNOT and GARROD in that it involved sequential steps in a reaction chain. Each step was determined by its own gene and, presumably, its own enzyme. This was a strong hint of things to come. Eventually, it led to the one-gene-one-enzyme hypothesis, fully documented in Neurospora. At the time, no more along this line could be done with Drosophila owing to the scarcity of nonautonomous mutants. Beyond that, however, BEADLE was conscious of the years of effort that he, TATUM, and many others had expended on the identification of the *vermilion* and *cinnabar* substances. A different, more effective way was needed. It occurred to him that "it ought to be possible to reverse the procedure we had been following and instead of attempting to work out the chemistry of known genetic differences we should be able to select mutants in which known chemical reactions were blocked. Neurospora was an obvious organism . . ." (BEADLE 1966). This idea—to search for nutritional (*i.e.,* nonautonomous) mutants in a genetically understood microorganism growing on a synthetic medium—was, as I have said more than once before, a stroke of genius: It created the science of biochemical genetics and made bacterial genetics possible.

In a curious article with some relevance to the present subject that has appeared in these pages, COMFORT (1995) gives a historian's view of the 1951 Cold Spring Harbor Symposium on *Genes and Mutations.* It is this author's belief that the 1951 Symposium was the occasion for a major confrontation between what he calls the MCCLINTOCK-GOLDSCHMIDT (or "dynamic") view of the gene and the BEADLE-TATUM (or "static") view. [In language and outlook, COMFORT's article strongly resembles EVELYN FOX KELLER's biography of BARBARA MCCLINTOCK (KELLER 1983).] COMFORT's picture of events rests, in part, on the fact that he misdates the acceptance by geneticists of the one-gene-one-enzyme idea. This idea, first intimated in the paper by BEADLE and EPHRUSSI commemorated here, was proposed formally by BEADLE in 1945, but it was not accepted by geneticists until they were compelled to do so by advances in the understanding of DNA and the genetic code made in the 'fifties and 'sixties. The Neurospora findings were widely admired, but the prevailing view in 1951 was that the conclusion BEADLE had drawn from them was a vast oversimplification. (This resistance was not found among microbiologists and biochemists, who welcomed the idea.) BEADLE (1966) wrote that after reading the 1951 Symposium volume, he had the impression that supporters of one-gene-one-enzyme "could be counted on the fingers of one hand, with a couple of fingers left over."

Unaware of the state of affairs, and apparently not noticing that the paper I gave at the Symposium (HOROWITZ and LEUPOLD 1951; HOROWITZ 1995) was a defense of one-gene-one-enzyme against its most influential critic—a Cold Spring Harbor frequenter named MAX DELBRÜCK—COMFORT assumes that BEADLE's hypothesis was already part of the entrenched genetic canon. This allows him to construct the scenario referred to above in which the "static" gene is opposed by the "dynamic" one. GOLDSCHMIDT's contribution to the "dynamic" gene at the 1951 Symposium consisted of some elegantly phrased but predictably implausible thoughts on genes and chromosomes, while MCCLINTOCK's was an account of her discovery of transposable elements.

COMFORT's article has a certain operatic quality, with arias and golden duets by MCCLINTOCK-GOLDSCHMIDT alternating with dark basso rumblings from the BEADLE-TATUM side, the villain's role being assigned to E. B. LEWIS. Unlike most operas, however, this one ends happily. "As is often the case," COMFORT says, "both sides were partly right." By this he means that MCCLINTOCK's findings were confirmed in diverse species, but transposable elements were found to encode proteins, just as do "static" genes. He does not tell us that no biological function has yet been established for these elements. It is still an open question whether, if one or all of an organism's transposable elements could be removed, the organism would be harmed or benefited. (A defensible guess is that the organism would benefit, but the

species would suffer from loss of a source of genetic variability.)

I attended McCLINTOCK's lecture in 1951. There was great interest in it. She gave it twice, owing to the fact that the small lecture hall was overfilled at the morning session. Before she began, M. DEMEREC, director of the laboratory, arose to announce that McCLINTOCK would repeat the lecture after dinner for those unable to find seats. I attended the evening session, heard the talk and, like many others, was mystified. I did not see BARBARA as a character in a drama, however, but as an old friend. We had first met in 1944, when BEADLE invited her to come to Stanford to straighten out the cytogenetics of Neurospora. She spent ten weeks in the lab, and I got to know her as a coworker. She was knowledgeable and intense, a perfectionist, with a sense of humor that revealed itself in a deep, hearty laugh, startling coming from a tiny woman. I can easily imagine her laughing that laugh on being told that 45 years after the 1951 Symposium, people would still be debating the significance of transposable elements and trying to find a role for them.

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